
EVOLUTION IN THE MADIINAE: EVIDENCE FROM ENZYME ELECTROPHORESIS

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ABSTRACT

The genetic differences observed among species contain historical information that is a function of their ancestry, mode of origin, and population structure over time. Studies of isozyme differentiation among the island species of *Dubautia*, *Wilkesia*, and *Argyroxiphium*, and among the mainland species of *Layia* are reviewed. Both groups provide examples of rapidly evolved progenitor-derivative species pairs as well as species showing gradual divergence as a function of their time of separation. A comparison of island and mainland taxa illustrates the effect of population structure on levels of genetic variability and the consequent effects on genetic differentiation among species. The colonization and speciation patterns within the Hawaiian archipelago can be inferred for some species pairs on the basis of shared unique alleles that are presumed to reflect a common ancestry. Gene duplications observed in the Madiinae suggest an ancient tetraploid origin for the Hawaiian species.

The Madiinae offer several advantages for the study of plant evolution. They are a cohesive, monophyletic group with a distinct biogeographic center of origin, the Pacific Coast of North America. The mainland genera inhabit most of the major community and habitat types within California. The Hawaiian genera have some of the most remarkably distinct morphologies found within the Compositae. Early work with the Madiinae included the classic studies of plant evolution done at the Carnegie Institution of Washington at Stanford (Clausen, 1962). *Layia* and *Madia* were used because they included species at various stages of evolutionary differentiation (Clausen, 1962). The Carnegie group worked on the assumption that the evolutionary record of a group of living species is reflected in the existing genetic relationships among species (Clausen, 1962). One of their primary means of assessing genetic relatedness was through experimental hybridization studies. Biochemical genetic techniques have provided new means of analyzing the number of genes shared by related species and have reduced the need to resort to time-consuming hybridization programs. Enzyme electrophoresis was the first technique that allowed the number of gene differences among species to be measured directly over a wide taxonomic range (Avise, 1974; Gottlieb, 1977). Modern molecular techniques promise to allow an even greater resolution of phylogenetic patterns and genetic divergence among species (Palmer, 1985). This pa-

per will review the isozyme data available in the Madiinae to illustrate how the pattern and degree of genetic differentiation can be used to make inferences on the mode and tempo of genetic divergence among species.

GENETIC VARIATION AND GENETIC DIFFERENTIATION AMONG SPECIES: MAINLAND TAXA

In a study of the genus *Layia*, Clausen et al. (1941) used extensive analyses of morphological characters, cytogenetics, and fertility relationships from interspecific hybridizations. They found that one group of six species formed a complex consisting of three morphologically discontinuous groups: *L. platyglossa*; *L. fremontii* and *L. chrysanthemoides*; and *L. jonesii*, *L. leucopappa*, and *L. munzii* (Clausen et al., 1941; Clausen, 1962). They proposed that these species had arisen allopatrically, through the gradual accumulation of many small genetic changes as a result of differential selective pressures in different environments. The morphologically similar species represented more recent stages in the divergence and accumulation of genetic differences among taxa (Clausen, 1962).

The degree of genetic relatedness and the pattern of genetic divergence predicted by the model of allopatric evolution was tested using allozymes. Warwick & Gottlieb (1985) examined the species

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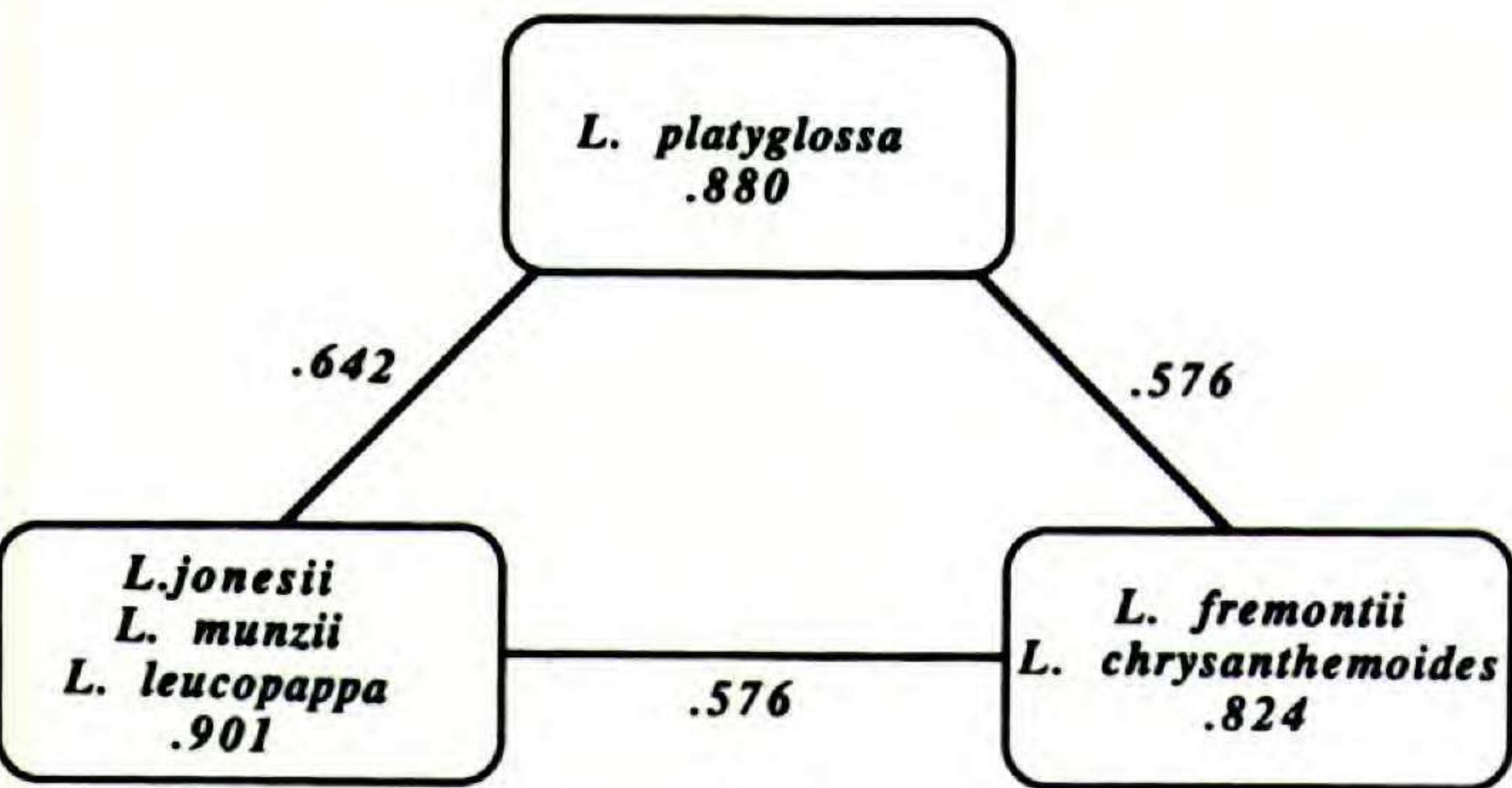


FIGURE 1. Summary of interpopulation and interspecific genetic identities (Nei, 1972) among the species of the *Layia platyglossa* complex. (Redrawn from Warwick & Gottlieb, 1985).

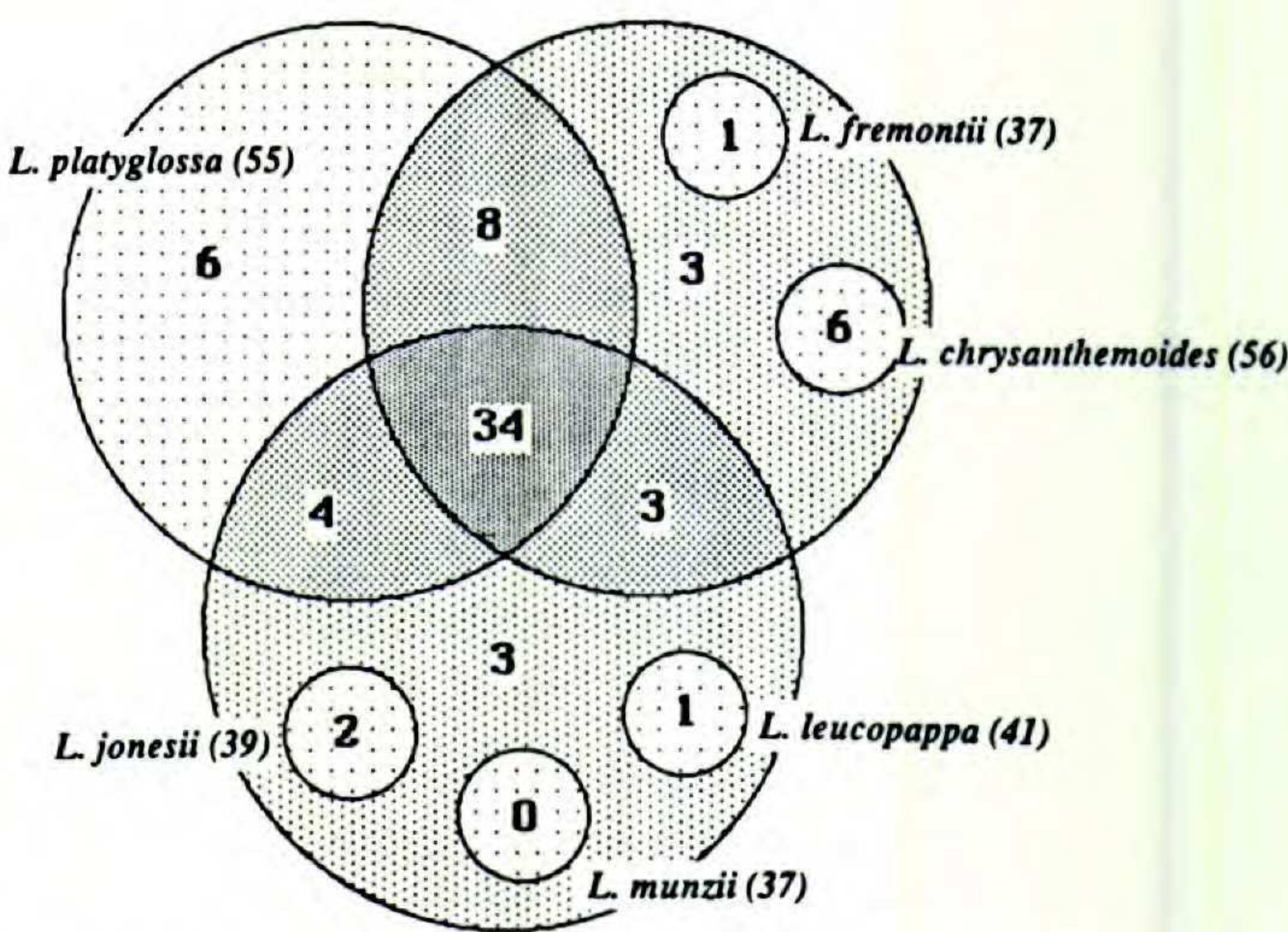


FIGURE 2. Numbers and distributions of individual alleles among the species of the *Layia platyglossa* complex. Total number of alleles identified per taxon is given in parentheses. Circles represent overlapping sets of individual species, individual species subgroups, and all three species subgroups of the complex. Numerals not in parentheses show the numbers of alleles that occur uniquely within individual species, within individual species subgroups, among any two subgroups, and the numbers of alleles that occur in all three subgroups (Gottlieb, unpublished data).

of the complex and found the genetic data to be fully concordant with an allopatric model of speciation (Fig. 1). Genetic distances were substantially higher among morphologically discontinuous species than among morphologically similar taxa. The pattern of allozymic divergence supports the concept of gradual genetic divergence through shifts in gene frequency. Figure 2 shows the distribution of alleles shared among taxa of the *L. platyglossa* complex. The vast majority of alleles are shared by either two or all three of the major species groups, while very few alleles are restricted to a single taxon or species group. All species of the complex are extremely polymorphic: an average of 67% of all loci are polymorphic per species, and the average number of alleles per locus is 3.4 (Warwick & Gottlieb, 1985). These data support the notion that allozymic divergence and genetic differentiation have occurred primarily as the result of changes in gene frequency from a rich, common pool of polymorphic gene loci. This pattern contrasts sharply with that observed in island populations.

ISLAND TAXA

The Hawaiian species of the Madiinae are renowned for their striking range of morphological and ecological diversification (Carr et al., 1988). The pattern of morphological and cytological differentiation is strongly correlated with the biogeographic distribution of species within the archipelago (Carr, 1978, 1985): many of the most morphologically specialized and differentiated species are endemic to the oldest island of Kauai and have the ancestral chromosome complement of 14 pairs of chromosomes. The cytologically most derived species, with 13 pairs of chromosomes, occur largely on the younger islands of Maui and Hawaii and are much less morphologically and cytologically

differentiated than the Kauai endemics. Estimates of allozymic divergence agreed with the hypothesis that the Kauai species were ancestral to the 13-paired species and that the 13-paired species were of relatively recent origin (Fig. 3; Witter & Carr, 1988). As in previous studies of island endemics (Crawford et al., 1985; Helenurm & Ganders, 1985; Lowrey & Crawford, 1985), the 13-paired species had extremely high interspecific genetic identities, $I > 0.90$. The 14-paired taxa, however, were significantly differentiated, with values more

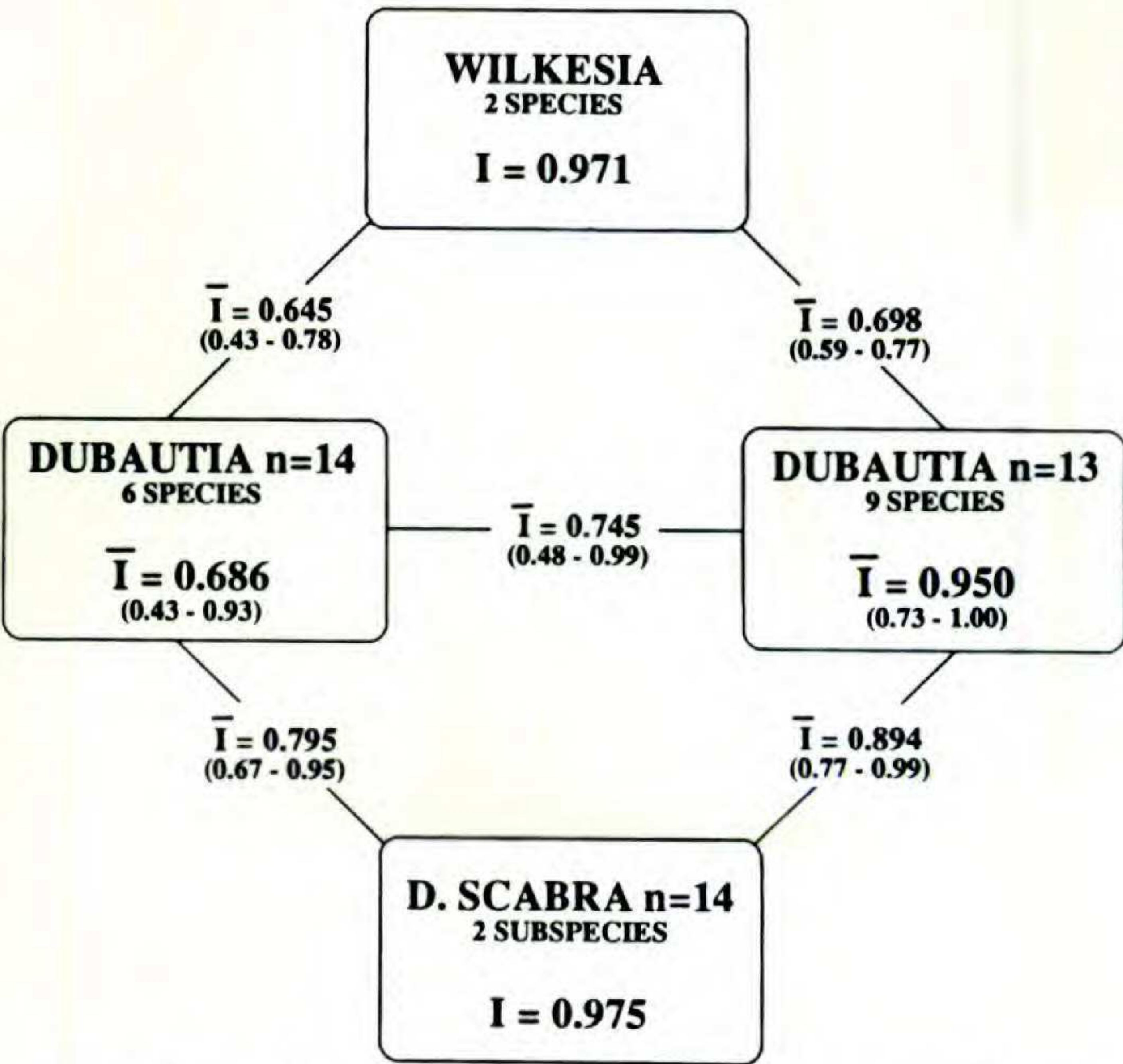


FIGURE 3. Summary of interspecific genetic identities (Nei, 1978) among the species of *Wilkesia* and *Dubautia*.

LOCUS: *Pgic 3*

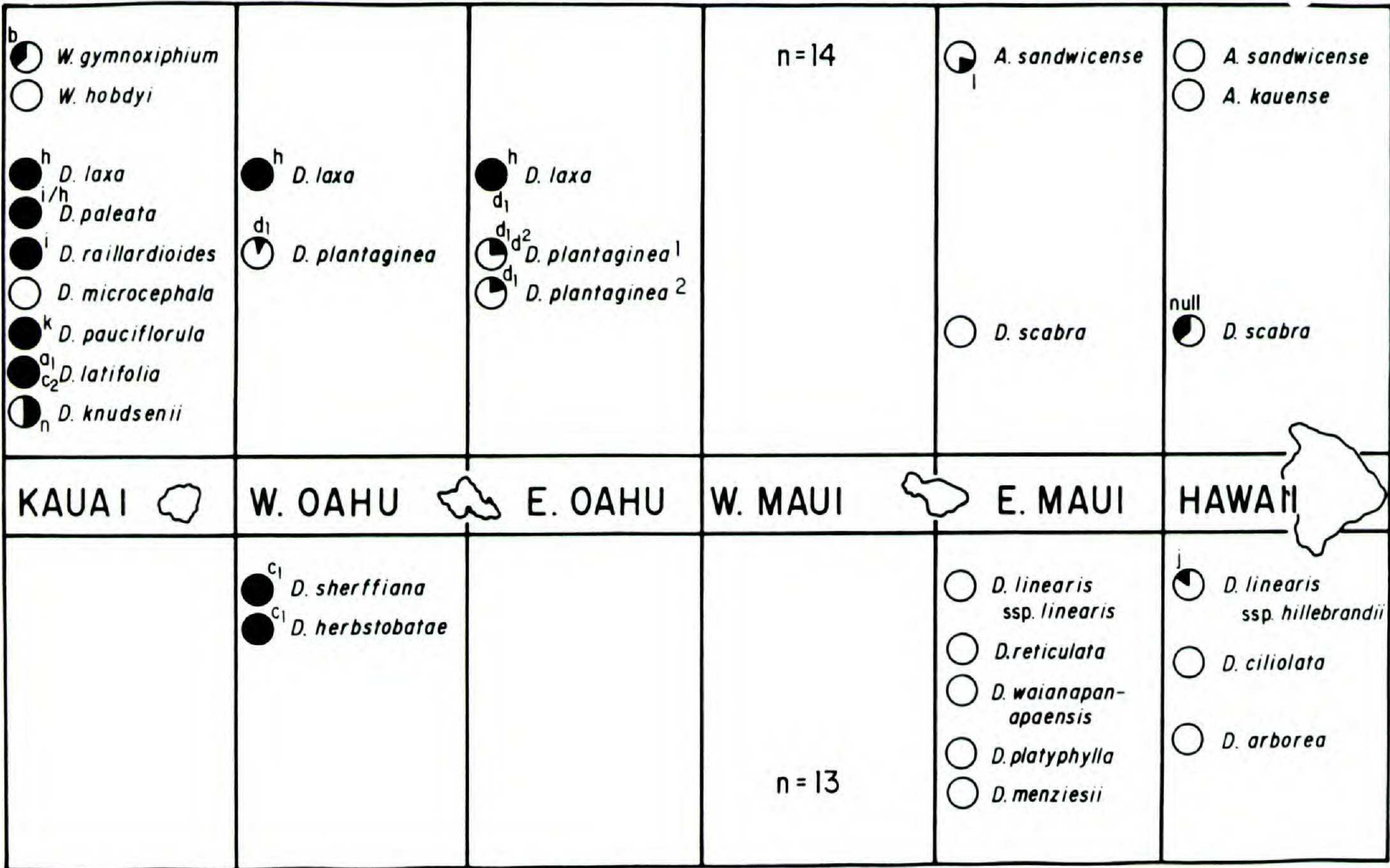


FIGURE 4. Distribution of allelic variants at the *Pgic 3* locus among the species of the Hawaiian Madiinae. The frequencies of particular alleles are illustrated in pie diagrams for each species. Open circles represent the frequency of a single, common allele that was found in a number of species. Closed circles represent the frequency of all other alleles found, most of which were restricted to one or two species.

typical of interspecific comparisons in other plant groups ($I \sim 0.67$, Gottlieb, 1981; Crawford, 1983). The results obtained for the 14-paired species of the Hawaiian Madiinae showed that insular plant populations do diverge genetically, but depend on sufficient lengths of time to accumulate genetic differences (Witter & Carr, 1988).

The pattern of interspecific genetic differentiation observed in island species appears to be the direct result of population structure. Low genetic variability in the Hawaiian Madiinae was attributed to founder events and genetic drift within small populations (Witter & Carr, 1988). Insular groups are frequently composed of small, localized populations with markedly decreased levels of genetic variation. For example, the Hawaiian Madiinae are approximately one-third as variable ($P = 0.24$, $A_i = 1.29$) as the species of *Layia* discussed previously. Figure 4 illustrates the typical distribution of alleles at a single locus among the species of the Hawaiian Madiinae and can be used to make several points about the effect of genetic variability on genetic divergence within the Hawaiian Madiinae. A single, common allele usually predominates at each locus among most species, suggesting a

genetically depauperate common ancestor. The low frequency of polymorphisms indicates that allozymic divergence will occur only as new mutants arise, rather than through gradual divergence in frequencies of multiple alleles at a given locus, as was observed in continental species of *Layia*. The distribution of alleles at *Pgic 3* (Fig. 4), for example, illustrates that the $n = 13$ species of *Dubautia* are all fixed for the same allele, which they presumably inherited from a single founder. The results of such a genetic bottleneck limit allozymic divergence among these taxa to the production and incorporation of new mutants. High genetic identities among the Hawaiian Madiinae are therefore not only a reflection of the length of time since divergence, but are also a function of the historical demography of species with consequent effects on genetic variability and structure of populations.

PROGENITOR-DERIVATIVE SPECIES PAIRS

Harlan Lewis was the first to propose the revolutionary notion of saltational speciation in annuals, whereby a new species could arise rapidly and be reproductively isolated from its progenitor

through the fixation of structural chromosome rearrangements (Lewis, 1973). Gottlieb (1981), in one of the early and striking systematic applications of enzyme electrophoresis in plants, showed that a number of species pairs believed to be related as progenitor and derivative had very high genetic identities and that the derivative species possessed a less variable subset of the alleles present in the progenitor. Recently derived species have historically been of interest to evolutionary biologists for several reasons (Ayala et al., 1975; Gottlieb, 1973, 1974; Carson, 1976). Better inferences can often be made about the recent past than about the distant past. Where polarity between species pairs can be identified, the direction of evolution in character change can be studied. Recent species pairs are also often partially or fully compatible, and interspecific hybrids can be analyzed for the genetic basis of species differences.

Layia discoidea is a highly localized, morphologically unusual species. Its most obvious morphological characteristics are an absence of ray florets with their enclosing bracts and a reduction of the pappus. Originally believed to be an ancient relictual form because of its highly distinctive morphology, experimental hybridization showed it to be fully interfertile with *Layia glandulosa* (Clausen, 1962). Subsequent allozyme analysis indicated that *L. discoidea* was a recent derivative of *L. glandulosa* subsp. *lutea* (Gottlieb et al., 1985). Unlike other annual progenitor-derivative species pairs, *L. glandulosa* and *L. discoidea* are fully interfertile and freely recombining. With their distinct morphological characteristics, they are ideal for the study of the genetics of characters often used to separate taxa at higher taxonomic levels (Gottlieb et al., 1985; Ford & Gottlieb, 1989).

The Hawaiian taxa also have a number of species pairs that fit the criteria of recent progenitor and derivative. Both species of *Wilkesia* are endemic to the island of Kauai. *Wilkesia gymnoxiphium* is a monocarpic rosette plant on an elongated woody stalk up to 3 m at reproductive maturity. It is widespread along the upper margins and outer slopes of Waimea Canyon. *Wilkesia hobydi* is much more restricted, known only from two populations on the sea cliffs of the Na Pali coast. Unlike *W. gymnoxiphium*, it is a multiply branched rosette plant which flowers repeatedly. The genetic identity between these two *Wilkesia* species is $I = 0.971$. At 8 out of 11 loci the two species share either identical monomorphic alleles or common polymorphisms, while at the remaining three loci *W. gymnoxiphium* is polymorphic and *W. hobydi* possesses only one of the alleles of the polymor-

phism (Witter, 1986). *Wilkesia hobydi* appears to be a recent derivative of the more widespread *W. gymnoxiphium* by the criterion of a reduced subset of genetic variability present in a derivative taxon. However, another possibility is that drift due to restricted population size could produce the decreased genetic variability in *W. hobydi*. Even if evolutionary polarity for the changes remains unsettled, the two species of *Wilkesia* offer an ideal opportunity to study the origin, ecological dynamics, and genetics of the complex life history trait of monocarpy.

Although all members of the group of 13-paired species of *Dubautia* have high genetic identities (Fig. 3), *D. dolosa* and *D. reticulata* appear to be a good case of a progenitor-derivative species pair. *Dubautia reticulata* is a large tree in the rainforests of east Maui, whereas *D. dolosa* is a rainforest shrub. The two species have a Nei's $I = 1.00$, sharing the same alleles at all loci. Several unique alleles are found only in these two taxa, supporting their common divergence from the other 13-paired taxa (Fig. 5). As with the two species of *Wilkesia*, the polarity of the relationship cannot be established on the basis of these allozyme data. Again, however, these species offer unique experimental opportunities. The distinct differentiation of life form in two species with a similar genetic background can be used to examine the genetic basis of the complex character trait of life form. Ecological hypotheses to explain the adaptive nature of life form differences suggest that trees in a tropical wet forest have evolved in response to shading and competition for light, while shrub forms are often associated with nutritional or other stresses (Rundel, pers. comm.). *Dubautia reticulata* and *D. dolosa* can be used to test the functional basis of the adaptational differences between trees and shrubs in this habitat.

DUPLICATE GENE EXPRESSION

Duplicate gene expression can result either from duplication of a single locus or through polyploidization. Unlinked single gene duplications may have originated in plants heterozygous for overlapping reciprocal translocations and have a high probability of being unique phylogenetic markers of shared ancestry (Gottlieb, 1986). Gottlieb (1986) documented two such unlinked duplications in the Madiinae: the first is a PGM duplication found throughout the diploid mainland genera; the second is an IDH duplication found throughout *Layia* (Fig. 6). A second IDH duplication occurs in two species of *Layia* with a cytotype of $n = 8$, and may prove

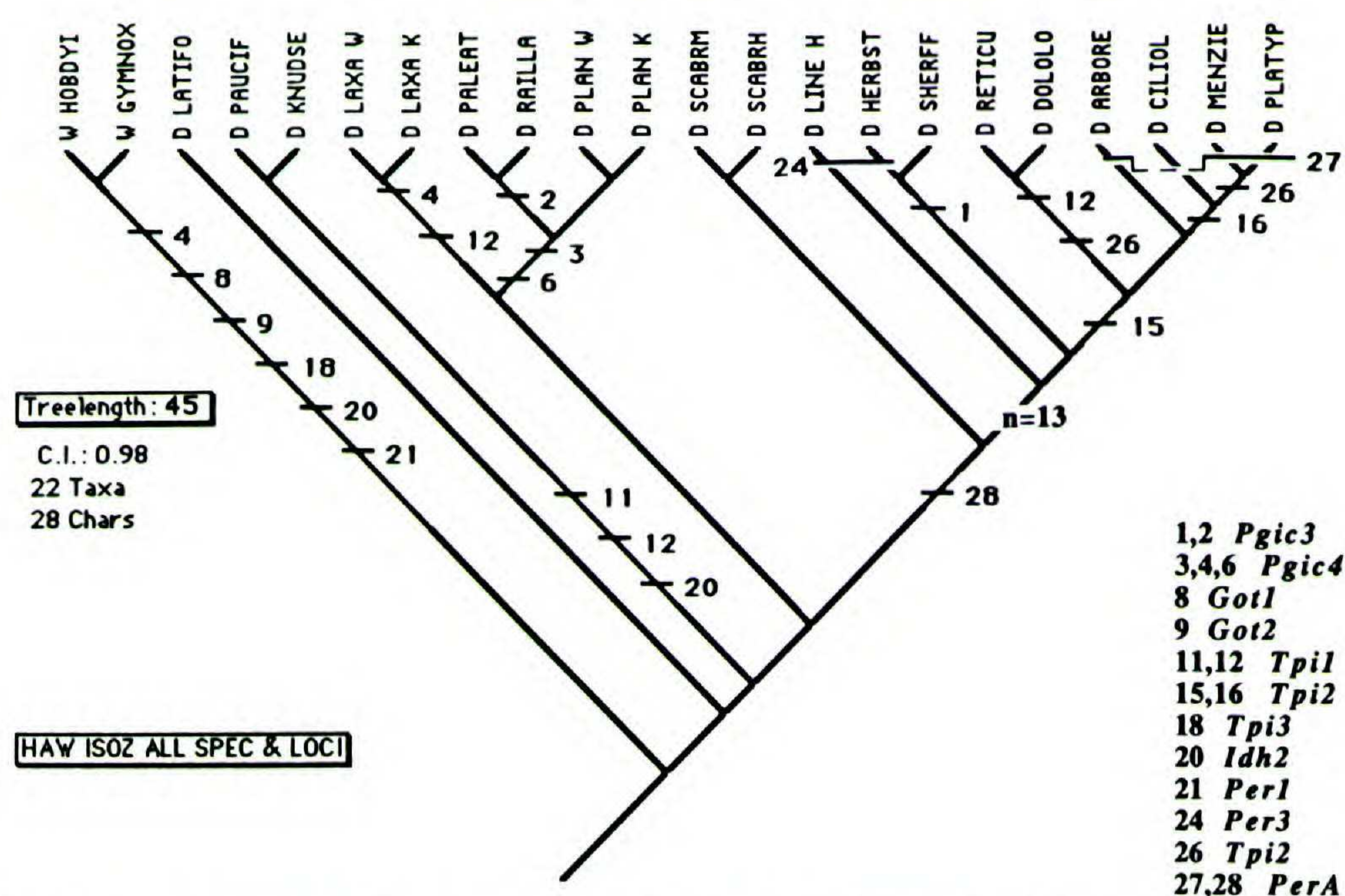


FIGURE 5. Distribution of unique alleles among the species of *Wilkesia* and *Dubautia*. The tree format was used to show the actual distribution of character states in the most consistent manner possible. The tree is arbitrarily rooted and polarity assumed and does not represent an actual phylogeny.

to be a phylogenetic marker for that section of *Layia* (Gottlieb et al., 1985; Fig. 6). Within *Layia* several other isozymic patterns are consistent with gene duplication, or could have arisen through other mechanisms, and must therefore be confirmed by genetic analysis. The LAP isozymes observed in the *Layia munzii* complex may be useful in resolving the trichotomy among the three taxa in this group (Fig. 6).

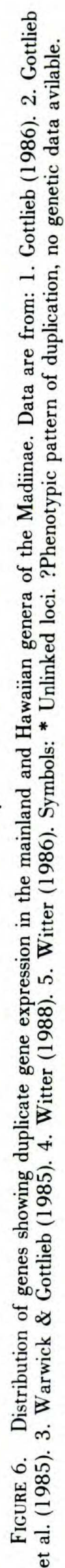
The Hawaiian Madiinae expressed three duplications in all taxa: PGMc, PGlc, and TPlc, and possibly a fourth locus, PGIp (Fig. 6). Because the base chromosome number in the Hawaiian Madiinae is $n = 14$, in a group where the ancestral condition is $n = 7$, it is assumed that these duplications result from polyploidization and are not multiple single gene duplications (Witter, 1988). This interpretation is supported additionally by the number of PGM loci expressed in the Hawaiian taxa. All Hawaiian species appear to express four PGM loci (Witter, 1988). The maximum number of expressed loci expected from an ancestral duplication in the Madiinae followed by polyploidization is four loci, the number observed. If the PGM loci were due to single gene duplications, it would be necessary to hypothesize two additional single gene duplications above the ancestral Madiinae PGM duplication.

The majority of 13-paired *Dubautia* species exhibited clear evidence of duplicated ADH activity, whereas there was no evidence of expressed duplications among the 14-paired species (Witter, 1988). The complex genetic model necessary to

explain the pattern of phenotypic variation of ADH isozymes of the Hawaiian Madiinae needs further genetic analysis; however, the 13-paired species are clearly differentiated in their pattern of duplicated ADHs (Fig. 7), and these may be a useful phylogenetic marker among these species which are otherwise difficult to differentiate cytologically or isozymically. Most interesting is the differentiation of the three subspecies of *D. linearis*, the most widespread and variable of the 13-paired taxa.

PHYLOGENETIC AND BIOGEOGRAPHIC INFERENCES FROM THE SYSTEMATIC DISTRIBUTION OF UNIQUE ALLELES IN THE HAWAIIAN TAXA

Argyroxiphium and *Wilkesia* each have a number of alleles that are unique to the genus and common to all taxa within the genus (Fig. 8). *Dubautia*, in contrast, has a large number of alleles unique to the genus, but these are largely restricted to single species or closely related species groups (Fig. 5). With the exception of a single PER phenotype common to *D. scabra* and the 13-paired species of *Dubautia*, and a single polymorphism, *Tpi2^a/Tpi2^d*, common to all lineages except *D. latifolia*, virtually no alleles define large clades within *Dubautia* (Figs. 5, 8). This suggests that some of the modern species of *Argyroxiphium* and *Wilkesia* are presumably much more recent than some of the lineages within *Dubautia*. In particular, the high genetic identity between the two



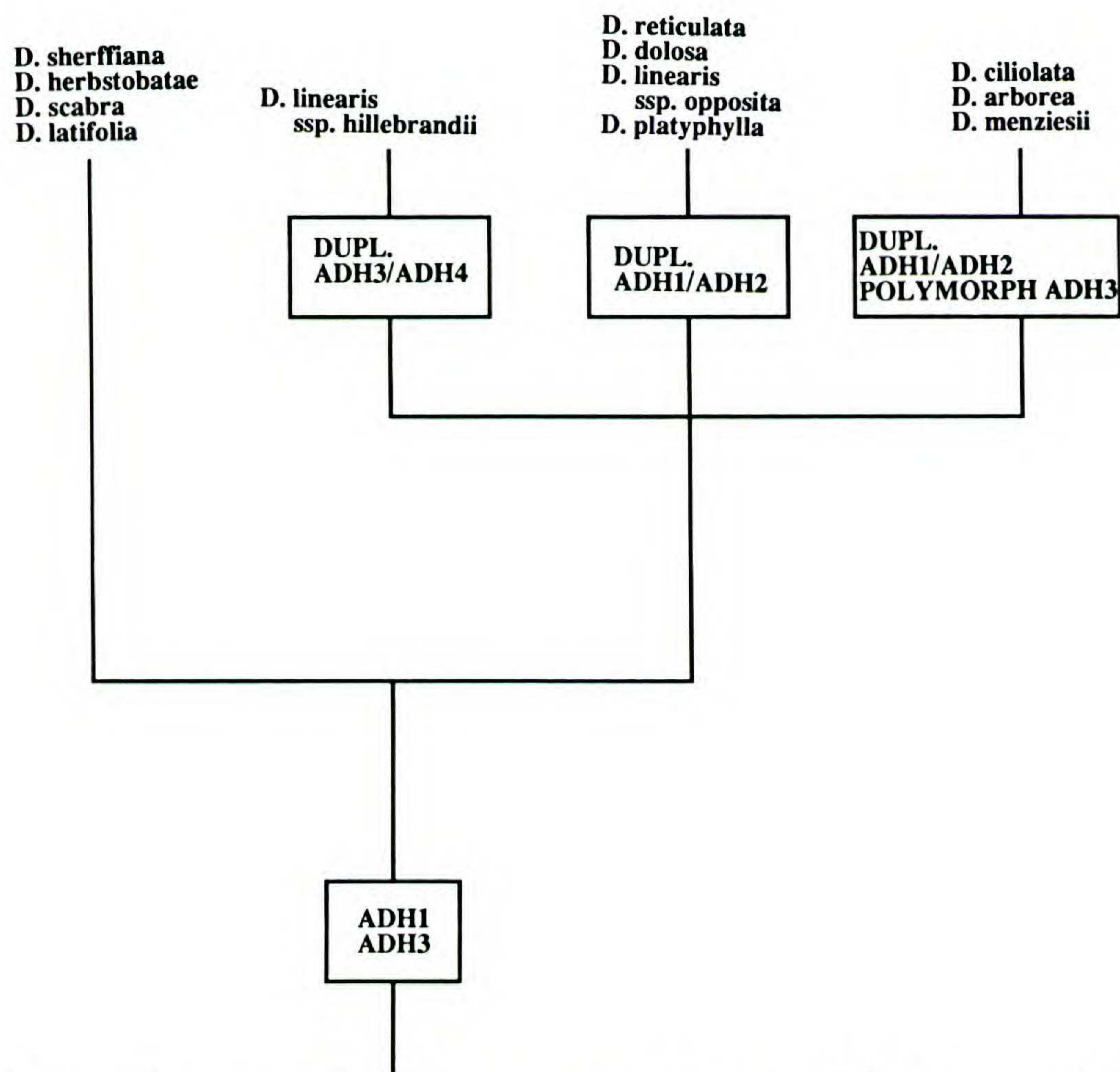


FIGURE 7. Differential expression of ADH isozymes among the species of *Dubautia* according to the genetic model of Witter (1988). All species are $n = 13$, except *D. latifolia* and *D. scabra*.

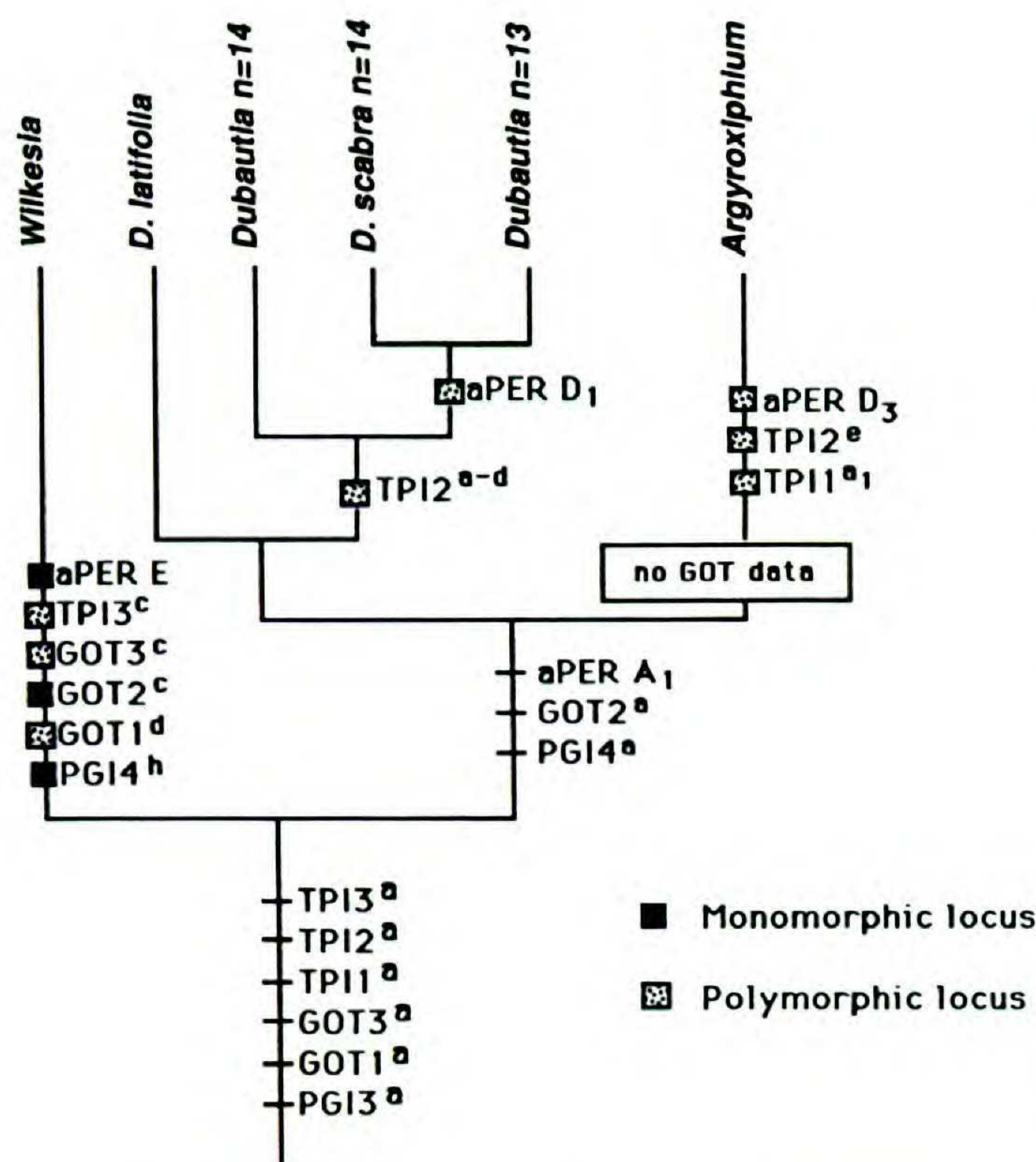


FIGURE 8. Distribution of unique alleles among the genera of the Hawaiian Madiinae. The tree format is used only to illustrate the distribution of alleles and does not represent actual phylogenetic relationships.

species of *Wilkesia* (Fig. 3) is significant to understanding the diversity within the Hawaiian Madiinae. Biogeographically and genetically (Fig. 5) the genus *Wilkesia* appears to be relatively ancient within Hawaii. However, speciation within the genus itself has been a much more recent phenomenon, and evolution even on the oldest major Hawaiian island, where *Wilkesia* is endemic, has not been static. The modern diversity of the Hawaiian Madiinae exists not only as the result of the accumulated products of ancient evolutionary events, but also because even the most ancient groups have continued to diversify and speciate.

In considering the distribution of unique alleles among the species of Hawaiian Madiinae, the shared alleles are generally taken to indicate common ancestry. However, accurately reconstructing phylogenetic relationships by the distribution of unique alleles is confounded by the fact that alleles originate in a polymorphic state and may persist for long periods as polymorphisms. Under these conditions the random loss of a synapomorphic allele may be fairly frequent, and true members of a clade may be excluded due to loss of alleles. Figure 5 illustrates just one of the possible tree topologies that can be generated using unique alleles with the

MacClade program (Maddison & Maddison, 1987). It shows that a single isozyme character is consistent with the cytological, morphological, and distributional data supporting *D. scabra* as the 14-paired species most closely related to the 13-paired taxa of *Dubautia*. Several species pairs that morphologically appear to be closely related are supported by the enzyme data: *D. reticulata* and *D. dolosa*; *D. sheriffiana* and *D. herbstobatae*; *D. paleata* and *D. raillardiodides*; *D. pauciflorula* and *D. knudsenii* (Fig. 5).

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